OBSERVATIONS ON THE RESISTANCE OF GRAIN WEEVILS TO CERTAIN FUMIGANTS

by

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PURPOSE

This work was undertaken to determine what factors are concerned in the resistance of grain weevils to fumigants. The two species of weevils known to regularly infest grain, the granary weevil (Sitophilus granarius Linn.) and the rice weevil (Sitophilus oryza Linn.) were used. The corn billbug (Sphenophorus callosus Oliv.) was used for some comparative histological studies.

Attention was chiefly concentrated upon the possible role of mechanical respiration in resistance. The action of the spiracles was given particular attention. The problem was attacked both from a physiological and a

histological standpoint. Certain peculiarities of resistance, apart from these considerations, were noted.

It is a pleasure rather than an obligation to acknowledge the help given me in this task by Dr. R. L. Parker and Dr. R. H. Painter. I am also indebted to Dr. R. C. Smith for various suggestions.

HISTORICAL INTRODUCTION

Although it long has been known that certain insects are resistant to killing by chemical substances, little effort has been made to determine what physiological factors influence and control this resistance. Endless tests have been made to discover the "percentage kill" obtained by the action of specific and proprietary compounds, with no consideration, apparently, of how these substances brought about the results. The same has also been true of studies of insect respiration; its relationship to insecticides has hardly been considered.

It was in 1904 that Ewing, while engaged in studies on Orthoptera, discovered that the mechanics of insect respiration is intimately associated with the segmental ganglia. In a paper as yet unpublished, I have also discussed this characteristic as it is found in the larvae of certain Anisoptera.

The most thorough studies on the relationship of poisons to insect physiology were made by Shafer, and were published in 1911 and 1915. Shafer used chiefly specimens of Passalus cornutus in his tests. He made complicated apparatus for the study of the physiological reactions of the beetles, and also performed numerous chemical tests. He discovered that the beetles were not easily killed by suffocation, and that the action of poisons could not be compared with the effects of suffocation. Moreover, there were no morbid changes in the tissues of treated insects until several hours after death. The poisons destroyed or greatly reduced the oxidases, reductases and catalases of the blood. Some of the fumigants rendered wax membranes less permeable to oxygen. Shafer concludes that the action of chemical poisons upon insects is purely physiological.

McIndoo (1916), experimenting with honey bees and with aphids came to the conclusion that nicotine sprays do not penetrate the tracheal system. He states that the nervous tissue of insects killed by nicotine resembles histologically that deprived of oxygen. In the same year Moore (1916) found that osmic acid does not enter the tracheae. Later (1917) he found that a large number of benzene derivatives are more toxic to house flies than

carbon disulphide. He states that the toxicity of these compounds is directly proportional to their boiling points.

While engaged with purely respiratory work, Krogh (1920) decided that there are two general types of insect respiration: that which takes place mostly be diffusion, and that aided by mechanical action. Insects depending upon the former type of respiration usually have a more rigid tracheal system than those making use of mechanical action. The bearing of this on the problem of resistance will be discussed later.

Bodine (1922) found that anaesthetics first increase, and later decrease, the carbon dioxide output of insects.

Back and Cotton (1925), after an extensive series of experiments with various volitile organic compounds, concluded that ethyl formate and ethyl acetate are very effective fumigants for the rice weevil. Impure ethyl acetate proved injurious to grain. It is interesting that these workers found a mixture of ethyl acetate and carbon tetrachloride a more effective fumigant than the ethyl acetate alone.

Lee (1925) found that the respiratory movements of <u>Melanoplus differentialis</u> and <u>M. femur-rubrum</u> follow a regular sequence, and that inspiration takes place chiefly

through the thoracic and first two pairs of abdominal spiracles, while expiration takes place through the remaining abdominal spiracles. Closure of the expiratory spiracles does not greatly affect the insect, but blocking of the inspiratory spiracles causes asphyxia. In general, the work of Wrede (1926) agrees with that of Lee. Wrede believes that the nervous control of respiration depends upon the carbon dioxide pressure.

Brinley and Baker (1927) tested the effect of both ethyl acetate and hydrocyanic acid upon various insects, including both the granary and rice weevils. They claim that the addition of ethyl acetate to hydrocyanic acid increases its toxicity. They add that temperature influences the toxic effect of hydrocyanic acid, but that humidity probably does not.

MATERIAL AND METHODS

The insects used in this work were the granary weevil (<u>Sitophilus granarius</u> Linn.), the rice weevil (<u>Sitophilus oryza</u> Linn.), and the corn billbug (<u>Sphenophorus callosus</u> Oliv.). The billbug was used only for the sake of making certain comparisons in connection with the morphology of the spiracles and associated structures.

The adult granary weevil varies in length from approximately two to three millimeters. It is about one millimeter in width. It is mahogany brown in color and can be distinguished superficially from the rice weevil by its polished surface. There are no metathoracic wings. The rice weevil is a little smaller than the granary weevil. The surface of the body is dull. Although each elytron bears a sub-basal and a sub-apical spot, these are not always distinct. Morphologically there is little difference between the rice and granary weevils. The corn billbug, although closely related to the above species, differs materially from them in structure. It is about one inch in length and a fourth of an inch in width; the color is black. All of these species have five pairs of abdominal spiracles. The grain weevils appear to have a pair of thoracic spiracles not present in the billbug. In all of these insects the proximal pair of abdominal spiracles are larger than the others. The abdominal spiracles lie in the pleural coria, between the highly chitinized ventrum and thinner dorsum of the abdomen. The thoracic spiracles appear to be located near the bases of the metathoracic legs.

My first experiment consisted in placing the beetles, first killed with ethyl acetate or hydrocyanic acid, in India ink under reduced pressure. The insects treated with the two fumigants were placed in separate vials containing ink, and these, in turn, were placed in a stout glass bottle. The bottle was then partially exhausted for five minutes, after that the beetles were removed and dissected.

The necessity for histological examination of the spiracles was soon evident. The beetles were dissected under fifty per cent alcohol with the aid of a binocular magnifier, and the dorsum removed. Usually the main tracheal trunks, with the associated spiracles, adhered to this. They were subsequently removed, passed through graded alcohols, and mounted. Various methods of treatment were tried. In some cases the soft tissues were left; in others these were removed with concentrated sodium hydroxide solution. Ehrlich's haematoxylin and eosin were used for staining the tissues.

In order to test the effect of ethyl acetate and hydrocyanic acid on the spiracles, beetles were treated with each of these substances until body movements ceased, after which they were dropped into warm Carnoy's solution. As a check, mounts were also made from beetles killed in

Carnoy's solution alone. It was thought that beetles so treated should show, when mounts were made, any difference that the fumigants might have on the action of the spiracles.

To verify certain anatomical data, as well as to determine what effect the fumigants might have on the tissues, some sections were made. Weevils deprived of the more chitinous parts were embedded in paraffin, cut into sections about thirty microns thick, and stained with iron-hematoxylin and Lichtgrün. Similar studies were made of the billbug, but only whole mounts were used.

The effect of poisons on the tracheal system of the living insects could be studied with the aid of a gas chamber devised for this purpose. The mechanism consisted of a glass ring mounted upon an ordinary glass slide with balsam and DeKhotinsky's cement. Two glass tubes, also cemented to the slide, communicated with this chamber. The top of the chamber was a loose square of glass held tight by rubber bands. The space between this and the glass ring was filled with vaseline. Gas was introduced into the chamber by means of the following apparatus: A flask was equipped with a rubber stopper through which passed a glass funnel and a glass tube. The stem of the

funnel reached nearly to the bottom of the flask. The glass tube communicated with a bottle containing the fumigant. This, in turn, connected with the gas chamber through a rubber tube fitted with a pinch-cock. Another rubber tube conducted the gas from the chamber to a bottle containing a little water; this tended to absorb the very poisonous hydrocyanic acid, and the passage of the gas through the liquid served to demonstrate that the apparatus was working properly.

In using this apparatus the pinch-cock was first closed, and the funnel filled with water. The insect to be studied, with elytra removed, was fastened in the chamber with paraffin. The glass square was then replaced and the whole chamber put under the binocular magnifier. A spot of intense light was concentrated on the insect. In this work a high power ocular and low power objective were used. The total magnification was eighty-four. The gas was allowed to enter the chamber by releasing the pinch-cock; thus causing the flow of water to force the gas into the chamber.

Finally, a series of tests were made to determine the comparative resistance of rice weevils to ethyl acetate and hydrocyanic acid. Ten beetles were used for

each test. They were placed in a small tube lightly plugged with cotton; this tube was placed in a larger vial containing calcium cyanide or a bit of cotton saturated with ethyl acetate. Careful watch was kept to determine just how long each insect continued to move. As soon as the last beetle had stopped, all were removed, and the time that elapsed between this removal and recovery was recorded.

RESULTS OF EXPERIMENTAL WORK

The results of ink injection are given in the following tables. Results were considered positive when the tracheal trunks of the abdomen, of which there are two, appeared black from injection ink.

TABLE I

Ink Injection After Treatment With Hydrocyanic Acid

Test	Number of Insects	Species	Results +	Results −
1	5	S. oryza	0	5
2	5	S. oryza	0	5
3	4	S. oryza	0	4
4	5	S. oryza	0	5
5	4	S. oryza	0	4
6	5	S. granarius	2	3
7	6	S. granarius	1	5
8	5	S. oryza	0	5
9	4	S. granarius	0	4
10	5	S. granarius	0	5
11	4	S. oryza	0	4
12	5	S. oryza	0	5
13	5	S. oryza	0	5
14	5	S. oryza	0	5

It is evident that the beetles treated with hydrocyanic acid were not readily injected. On the other hand, many treated with ethyl acetate did show some tendency in ink (Table II).

TABLE II

Ink Injection After Treatment With Ethyl Acetate

Test	Number of Insects	Species	Results +	Results −
1	7	S. oryza	5	2
2	5	S. oryza	3	2
3	5	S. oryza	4	1
4	1	S. granarius	0	1
5	5	S. oryza	3	2
6	4	S. oryza	2	2
7	5	S. oryza	3	2
8	5	S. granarius	0	5
9	5	S. granarius	1	4
10	4	S. oryza	3	1
11	5	S. oryza	3	2
12	4	S. granarius	0	4
13	5	S. oryza	3	2
14	5	S. oryza	4	1

The results seemed to indicate a possible action of the spiracles in excluding certain fumigants. Histological data, however, did not justify such a conclusion. In fact, none of the spiracles appeared to be closed at any time, no matter how the insects were killed. This

condition was not affected by histological technique. The chitinous bow associated with the spiracles of the granary and rice weevils does not seem fitted to act as an occluding apparatus.

On the other hand, the billbugs, which have a rather intricate occluding apparatus, always had the tracheae closed after death. This closure was also apparently uninfluenced by the way in which the beetles were killed. The openings were quite as tightly closed in those treated with ethyl acetate as in those treated with hydrocyanic acid.

Examination of living insects in the gas chamber did not reveal much that might not have been predicted. Not only were the spiracles visible; the tracheae were observable through the transparent dorsum. No particular action of the system was noted. The chitinous bow did not appear to move a great deal, and the movements that did occur might easily have been due to movements of the body fluids. It was noted that an unfolding of the plural coria and compression of the dorsum tended to shut off the air supply to the tracheal system. Insects subjected to hydrocyanic acid quickly stopped all respiratory movements, while those treated with ethyl acetate continued these movements for some time.

The results of the resistance of the rice weevil to hydrocyanic acid are given in Table III. Lack of material and time prevented the repetition of these tests on the granary weevil.

TABLE III

Resistance of the Rice Weevil to Hydrocyanic Acid

Insect	Movements ceased	Movements resumed
1	In 1 minute	In 2 minutes
2	In 2 minutes	In 2 minutes, 30 seconds
3	In 2 minutes	In 3 minutes
4	In 2 minutes	In 3 minutes
5	In 3 minutes	In 5 minutes
6	In 3 minutes	In 9 minutes
7	In 3 minutes	0
8	In 4 minutes	0
9	In 4 minutes	0
10	In 3 minutes	0
11	In 1 minute	0
12	In 1 minute	0
13	In 1 minute	0
14	In 2 minutes	0
15	In 2 minutes	0
16	In 2 minutes	0

TABLE III Cont'd

Insect	Movements ceased	Movements resumed*
17	In 2 minutes	0
18	In 3 minutes	0
19	In 3 minutes	0
20	In 3 minutes	0
21	In 30 seconds	In 10 minutes
22	In 30 seconds	In 20 minutes
23	In 1 minute	In 30 minutes
24	In 1 minute	In 33 minutes
25	In 5 minutes	0
26	In 5 minutes	0
27	In 5 minutes	0
28	In 5 minutes	0
29	In 5 minutes	0
30	In 5 minutes	0

*The ciphers in the third column indicate either that the insects did not recover, or that they recovered only after a period of twenty-four hours.

Treatment with ethyl acetate gave the results indicated in Table IV.

TABLE IV

Resistance of the Rice Weevil to Ethyl Acetate

Insect	Movements ceased	Movements resumed
1	In 7 minutes	In 12 minutes
2	In 7 minutes	In 12 minutes
3	In 7 minutes	In 13 minutes
4	In 7 minutes	0
5	In 10 minutes	0
6	In 12 minutes	0
7	In 12 minutes	0
8	In 12 minutes	0
9	In 12 minutes	0
10	In 14 minutes	0
11	In 2.5 minutes	In 9 minutes
12	In 2 minutes	In 17 minutes
13	In 3 minutes	In 18 minutes
14	In 3 minutes	0
15	In 3 minutes	0
16	In 3.5 minutes	0
17	In 4 minutes	0

TABLE IV Cont'd

Insect	Movements ceased	Movements resumed
18	In 4.5 minutes	0
19	In 4.5 minutes	0
20	In 7.5 minutes	0
21	In 2 minutes	In 8 minutes
22	In 2 minutes	0
23	In 2 minutes	0
24	In 2 minutes	In 10 minutes
25	In 4 minutes	0
26	In 4 minutes	0
27	In 4 minutes	0
28	In 4 minutes	0
29	In 5 minutes	0
30	In 5 minutes	0

These results justify the conclusion that there is actually little difference in efficiency as killing agents between hydrocyanic acid and ethyl acetate. Any difference that exists favors ethyl acetate as a killing agent.

DISCUSSION

Morphologically the tracheal system of the grain weevil is of great interest. It is essentially the same in both the rice and the granary weevil. The more important parts are the large tracheal trunks which lie in either side of the body cavity, the spiracles, and the structures associated with the spiracles. In the thorax the tracheal trunks are large, single, and many-branched. Each abdominal trunk consists of a large ventral and a smaller dorsal trachea. These tracheae are fused at the places where they communicate with spiracles. The spiracles proper consists of an oval opening with a more or less chitinous margin. This is represented by "S" in Plate II. From this a tube (A) connects with a globular vesicle or chamber (V). At the point of union the trachea is much reduced in diameter. It is also characterized by the presence of a chitinous bow (B), which seems to be an integral part of the tracheal wall. I have not been able to determine that this bow serves any purpose, excepting a possible support for this part of the trachea. Inasmuch as the whole tracheal system of these insects is quite rigid, this function seems superfluous. Indeed

the rigidity of the system indicates a respiration largely dependent upon diffusion. In the drawing, T and T_1 indicate respectively the dorsal and ventral branches of the main tracheal trunk.

Another point that is difficult to determine is the exact nature of that part of the trachea lying between the spiracle proper and the chitinous bow. Its position suggests an atrium, but it differs from an atrium in various ways, especially in having tenidia. Imms (1924) states that the atrium regularly "lacks tenidia" (p. 108). Either this is an exception to Imms claim, or what seems more likely, the atrium proper has disappeared.

The status of the chitinous bow is made somewhat clearer by the examination of the spiracular appurtenances of the billbug. In this species the spiracle is slightly heart-shaped, and there is a true atrium. The well-developed closing apparatus consists of two triangular pieces with two of their shorter sides approximated, and hinged at the appressed apices. The free apices of the triangles are curved inward and connected by a band of muscles. Between the opposed faces of the triangles is a slit which is opened or closed by the action of these muscles. Long trident hairs interlace across the opening.

On the inner surface, this occluding apparatus communicates with a large abdominal air-sac; typical in being without tenodia.

In keeping with what has been stated by Krogh (1920) in respect to the comparative rigidity of the tracheal system, it appears that the smaller rigid system of Sitophilus granarius and S. oryza are correlated with a respiration by diffusion, and that the system found in Sphenophorus callosus is related to mechanical breathing systems probably necessary for so large an animal.

The same might be said of the closing apparatus. The bow found in the grain weevils may be a degenerated occluding mechanism. It has no muscles to bring about occlusion, and it has lost its intimate connection with the body wall. The atrium seems to have disappeared. That these beetles are undergoing degenerative changes is very probable. The granary weevil has already lost its wings.

It is to be doubted if the difference in action of fumigants is mechanical. It may be that the cessation of breathing movements caused by hydrocyanic acid restricts poisoning to that of slow diffusion. It is also possible that ethyl acetate acts differently upon the nervous system than does the hydrocyanic acid. Since the

respiratory movements are controlled by the segmental ganglia, this may be more significant than it appears.

The histological sections did not show any degeneration of tissues.

CONCLUSIONS

1. Ethyl acetate is slightly more effective than hydrocyanic acid gas in killing the granary weevil (Sitophilus granarius) and the rice weevil (S. oryza).

2. Ethyl acetate does not greatly change respiratory movements, while hydrocyanic acid tends to suspend or stop these movements.

3. There is no apparent degeneration of tissue resulting from treatment with either of these fumigants.

4. The resistance of the grain weevils does not depend upon the action of an occluding apparatus. The chitinous bows associated with the spiracles appear to be functionless.

5. The chitinous bows may represent a stage in the degeneration of the functional occluding apparatus found in the billbug (Sphenophorus callosus).

6. Resistance may be dependent upon some deep-seated physiological condition, or it may be a matter of rate of diffusion.

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EXPLANATION OF PLATES

PLATE I

Gas Chamber

A, tube for entrance of gas.

B, tube for escape of gas.

C, glass slide, upon which parts are mounted.

D, glass ring, forming chamber.

E, glass square used to cover chamber.

F, insect fastened in paraffin block.

G and G_1, rubber bands holding glass cover in place.

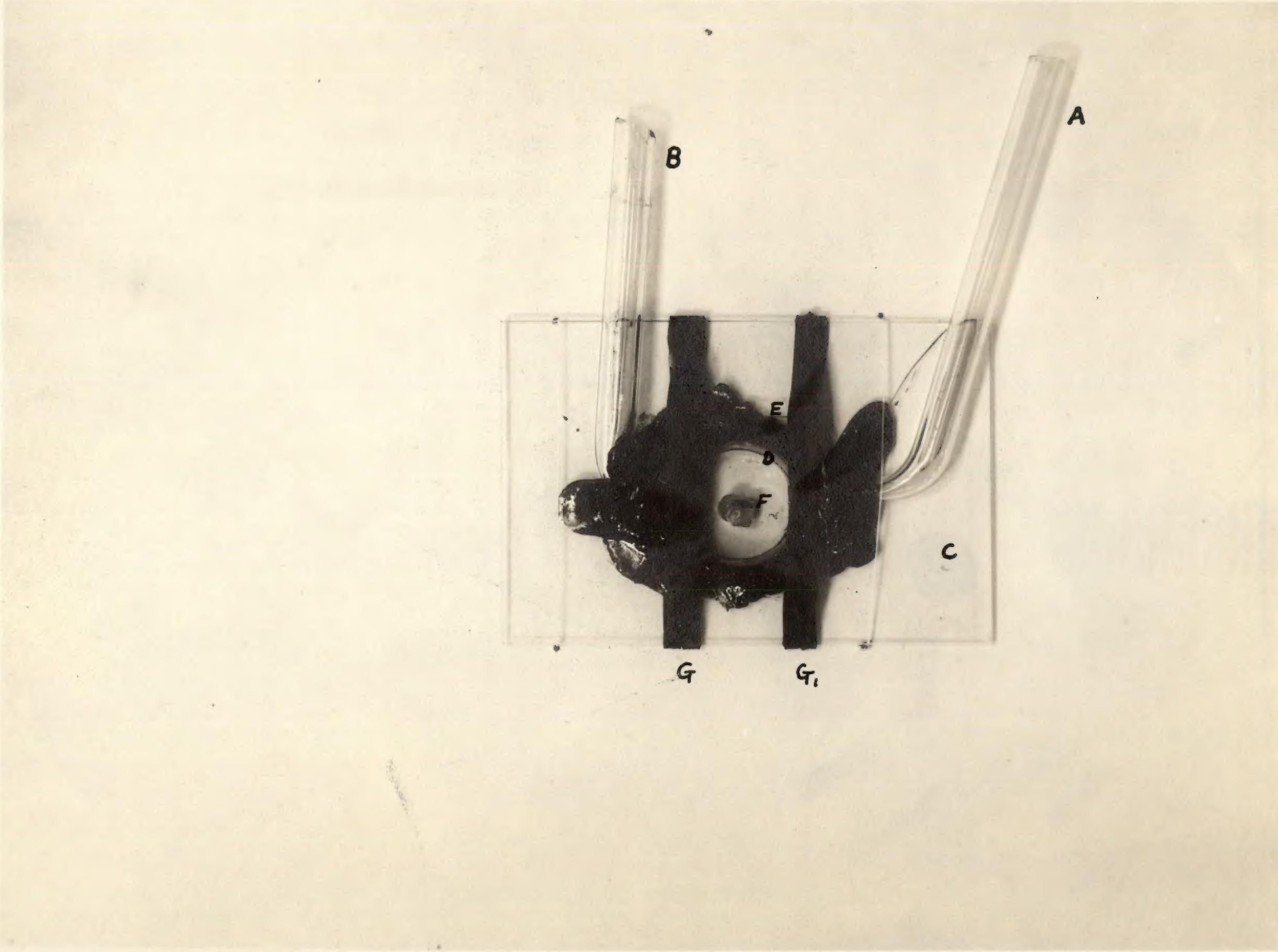

Plate I

PLATE II

Spiracle and Associated Structures of

Sitophilus granarius Linn.

S, spiracle.

A, trachea between spiracle and bow.

B, chitinous bow.

V, vesicle or inner chamber.

T and T_1, the dorsal and ventral branches of the principal trachial trunk.

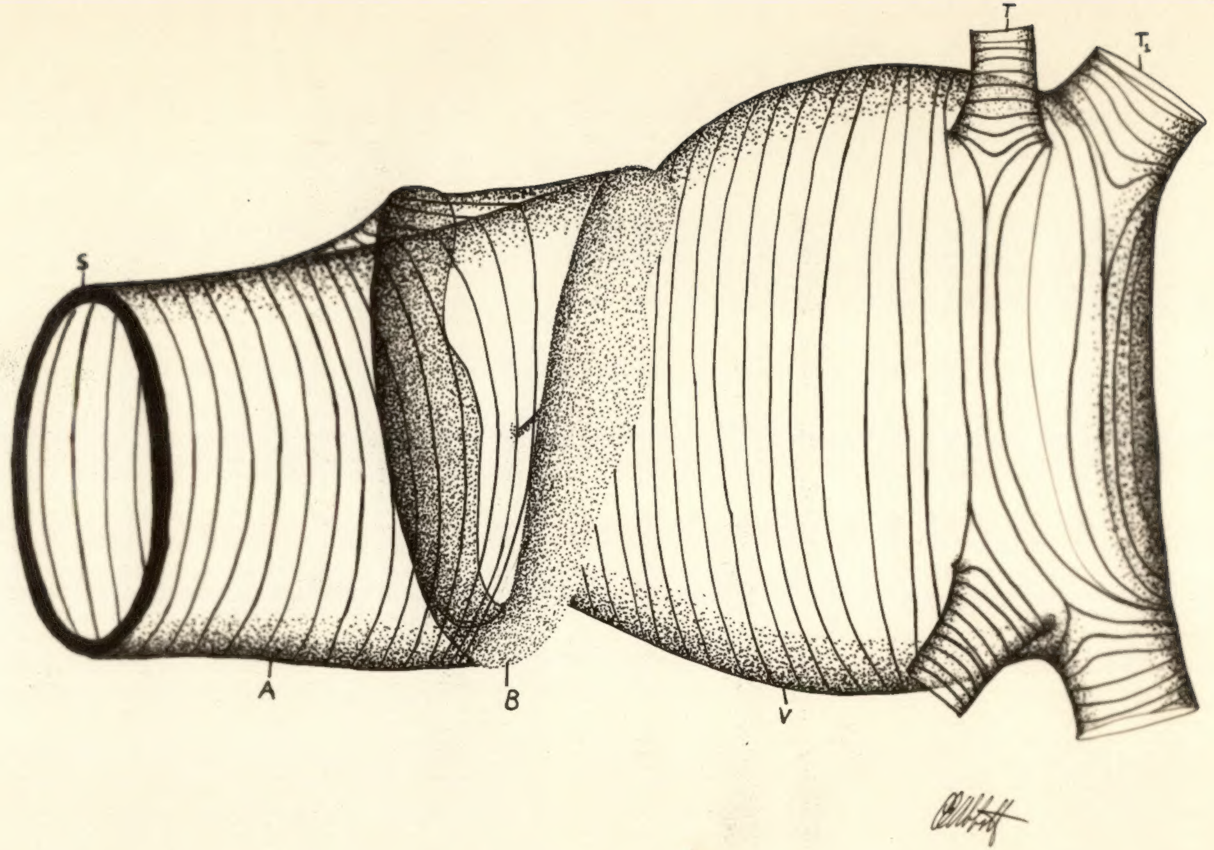

Plate II

Date Due
